Remarks

The request for a sequence ID is noted and has been communicated to the client in the United Kingdom. The sequence ID will be provided in a supplementary amendment upon clarification from the inventor.

Priority

The Examiner has requested a certified copy of the priority application but as this application is based on a PCT application an additional priority filing is not required. (MPEP 201.11(a))

Utility

The claims now relate to a method for delivering a peptide into the major histocampatability complex (MHC) class I antigen processing pathway of an antigen presenting cell to elicit a cytotoxic T lymphocyte (CTL) response. The method involves the step of contacting the cell with a mutant EtxB or CtxB covalently linked to the peptide.

The claim does set out the steps involved in the method.

Enablement

The claims are now limited to the use of EtxB/CtxB having a mutation in a particular 8 amino acid segment (the β4-α2 loop) to deliver a peptide to the MHC class I processing pathway of an antigen presenting cell. The experimental data in the application as filed show that contacting one of the EtxB/CtxB mutants carrying a class I epitope with an antigen presenting cell can be used to deliver the epitope into the class I antigen presentation pathway. It makes no difference whether the antigen-presenting cell is in a tissue culture tray or in a whole animal, since it is the function of antigen presenting cells to sample antigen and all antigen presenting cells express GM1. It is to be noted that

the data in the application as filed were obtained using living cells; not a test tube cell free system of vesicles.

Applicant gratefully acknowledges that the Examiner has indicated that the specific mutants of EtxB (CtxB)(E51A), CtxB(Q56A), CtxB(H57A) and EtxB(H57S) are enabled. Applicant respectfully submits that it is reasonable to extend this to the eight amino residues in the E51-I58 section of the β4-α2 loop. As explained in WO/14114 (page 13, lines 19-25) the present inventors believe that the β4-α2 loop of ExtB/CtxB mediates a low affinity "secondary" binding activity which occurs after the high affinity GM-1 binding reaction. It is thus reasonable to predict that, as a mutation in H57 of EtxB or CtxB produces a molecule capable of binding GM-1 but lacking the ability to trigger signalling events in the leukocytes, mutation of any of the E51-I58 residues of the β4-α2 loop would produce a molecule having similar properties.

The examiner argues that the claim relates to:

"A method of delivering <u>any</u> agent to <u>any</u> target cell comprising contacting said cell with <u>any</u> mutant of EtxB or <u>any</u> mutant of CtxB, thereby delivering said agent to said cell resulting in treatment of <u>any and all</u> disorders and diseases."

However, the amended claims do not read as suggested by the Examiner. The amended claims require that :

(i) the <u>agent</u> is a peptide covalently linked to a mutant form of EtxB or CtxB;

(ii) the <u>target cell</u> must be an antigen presenting cell expressing GM-1 ganglioside receptors on its surface;

(iii) the <u>mutant</u> must be in the eight amino residues of the E51-I58 section of the β4-α2 loop (see above);

(iv) the method is to deliver a peptide into the MHC class I antigen processing pathway of an antigen presenting cell to elicit a cytotoxic T lymphocyte (CTL) response, not to treat a disorder or disease.

We submit that the specification reasonably provides enablement for this subject-matter.

Claims relating to a method for treating a disease (previous claims 14 to 16) have been canceled.

Indefiniteness

It is submitted that the amended claims are clear and definite as to what method/process the applicant is intending to encompass (i.e. a method for delivering a peptide into the MHC class I antigen processing pathway of an antigen presenting cell to elicit a CTL response) and the steps involved in the method (i.e. contacting the cell with a mutant EtxB or CtxB covalently linked to the peptide).

Novelty

WO 00/14114 teaches that EtxB molecules with point mutations in the β4-α2 loop retain GM-1 binding activity, but lack other activities such as toxicity and the capacity to upregulate CD25 and trigger apoptosis of CD8-positive T cells (see page 12, lines 20-24). However, this document does not teach or suggest that a mutant form of EtxB or CtxB having a mutation in the β4-α2 loop can be used to deliver an exogenous peptide into the MHC class I antigen processing pathway of an antigen presenting cell to elicit a CTL response. The subject matter of the present claims is therefore novel over the teaching of this document.

Obviousness

Applicant submits that there is nothing in the other documents cited by the examiner which would induce the skilled person to use the mutant forms of EtxB or CtxB described in WO 00/14114 to deliver an exogenous peptide into the MHC class I antigen processing pathway of an antigen presenting cell to elicit a CTL response. The references are soecifically discussed as follows.

Loregian *et al*

As mentioned by the examiner, Loregian *et al* teaches that a peptide, when fused to CtxB/EtxB, mediates the delivery of the fusion protein into the nucleus of cells when the fusion protein is incubated with the cells. It does not teach or suggest that the peptide may be delivered to the MHC class I antigen processing pathway of an antigen presenting cell.

The nucleus of the antigen presenting cell is not involved in the MHC class I antigen processing pathway. As shown in the enclosed excerpts from "Immunobiology" (Janeway and Travers published in 1994 by Current Biology Limited) in the normal course of events, cytosolic pathogens are degraded in the cytoplasm of an antigen presenting cell (see figure 4.2 on page 4:3) Peptides generated by degradation of proteins in the cytoplasm are then transported into the lumen of the endoplasmic reticulum where they bind MHC class I molecules. The MHC: peptide complex is then transported through the Golgi complex to the cell surface (see figure 4.11 on page 4:11). Loregian et al therefore teaches away from any suggestion that EtxB/CtxB or mutants thereof can be used to deliver a peptide to the MHC Class I antigen processing pathway.

The statements made on the last paragraph of page 5226 are purely speculative. As mentioned above this paper reports the intranuclear delivery of an antiviral peptide. The last paragraph speculates that EtxB-based chimeras maybe engineered "by introduction of specific signals into attached peptides to either enhance or prevent cleavage, potentiate or inhibit insertion across endocytic membranes, and ensure targeting to other in-

tracellular compartments, such as Golgi apparatus, endoplasmic reticulum, or mitochondria".

There is absolutely no discussion as to what these "specific signals" may be that would potentiate insertion to compartments other than the nucleus.

Marcello *et al*

The non a peptide YAGAVVNDL is known to specifically inhibit ribonucleotide reductase from herpes simplex virus *in vitro*. Marcello *et al* show that EtxB can be used as a carrier for delivery of this nonapeptide into virally infected cells. The aim of this document is therefore to inhibit HSV replication in cells. From the discussion section of Marcello et al, it is clear that it is not known exactly how the EtxB- R2 complex acts in infected cells (see paragraph spanning pages 8997 and 8998). It is thought that it may be internalised and delivered to a site where the nonapeptide can compete with R2 for the binding site on R1. This may require translocation of the complex into the "cytosol or nucleus" where HSV-RR maybe located. At best, Marcello *et al* therefore teaches that EtxB conjugate can access a compartment of a virally-infected cell where HSV-RR may be located. It does not teach or suggest that EtxB would be capable of delivering an exogenous peptide into the MHC class I antigen processing pathway of an antigen presenting cell. It is to be noted that the cells described in Marcello *et al* (Vero cells) are from a kidney cell line, and are not antigen presenting cells.

Nashar et al [A] and [B]

Nashar *et al* [A] reports that EtxB can be used for the oral delivery of heterologous antigens and epitopes in particular to the gut mucosa. It does not teach or suggest that EtxB, or mutants thereof, can be used to deliver exogenous peptides into the MHC class I antigen processing pathway of antigen presenting cell.

Nashar *et al* [B] also teaches away from the present invention. Nashar *et al* [B] investigates the role of GM-1 in antigen presentation by MHC class II molecules by comparing the affects of EtxB versus EtxB (G33D), a variant which does not bind GM-1. They demonstrate that the binding of EtxB to the antigen presenting cell directly augments the expression of MHC class II on B cells, and fractionation of B cells demonstrated that EtxB binding to GM-1 results in rapid internalisation and targeting to Class II-rich compartments (penultimate line of abstract). This would suggest that EtxB maybe used to deliver peptides to the MHC class **II** antigen processing pathway of an antigen presenting cell, rather than the MHC class I antigen processing pathway. It suggests, as EtxB binding to GM-1 increases expression of MHC class II on B cells, that it would shift the response towards an MHC class II type response. Nashar et al B does not teach or suggest that EtxB or a mutant thereof could be used to deliver a peptide to the MHC class I antigen processing pathway of an APC to elicit a CTL response.

In view of the above, we submit that it is impermissible to combine the documents cited by the examiner and even if the skilled person were to do so, they would not arrive at the subject matter of the present invention.

Conclusion

For all the foregoing reasons, it is respectfully submitted that claims 1-3, 10-12, 17-18, and 21, and 26, all of the claims remaining in the application, are enabled; specific and definite; and patentable over the art of record. It is respectfully requested that a Notice of Allowance be issued in this application.

Respectfully submitted,

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